Flavin-oligonucleotide conjugates: sequence specific photocleavage of DNA

Christelle Frier,[a] Jean-François Mouscadet,[b] Jean-Luc Décout,*[c] Christian Auclair[b] and Marc Fontecave*[a]

[a] *Laboratoire de Chimie et Biochimie des Centres Redox Biologiques, DBMS-CEA/CNRS/Université J. Fourier, Bat. K, 17 avenue des Martyrs, 38054 Grenoble Cedex 9, France. E-mail: fontecav@cbcrb.ceng.cea.fr*

[b] *CNRS UMR 1772, Institut Gustave Roussy, PRII 39, rue Camille Desmoulins, 94805 Villejuif Cedex, France*

[c] *Laboratoire de Chimie Bioorganique, CNRS EP 811, UFR de Pharmacie, Domaine de la Merci, 38706 La Tronche, France. E-mail: decout@cbcrb.ceng.cea.fr*

Received (in Cambridge, UK) 4th August 1998, Accepted 6th October 1998

A flavin–oligonucleotide conjugate forms a stable triple helix with a double-stranded DNA sequence of HIV-1, and selectively photocleaves it at the 3′-G of a GG doublet located 7 bases away from the flavin position.

Oligonucleotides can be used as selective inhibitors of gene expression.[1] Binding to double-stranded DNA results in short triple helix formation which can block transcription.[2] Moreover, attachment to the oligonucleotide of a reactive group that can irreversibly damage the target DNA in a site-specific fashion should potentiate the inhibitory properties of the oligonucleotide.[2,3]

As a substituent of triple helix forming oligonucleotides (TFOs), flavins such as riboflavin **1** (vitamin B2, Fig. 1) have the advantage of displaying low toxicity and might be activated *in vivo* by diaphorases to produce oxygen species to damage a DNA target.[4] Flavin–TFOs might also be useful tools for *in vitro* studies: riboflavin is one of the most efficient natural photosensitizers, and has previously been shown to induce DNA oxidation[5] and selective RNA cleavage.[6] Upon irradiation, netropsin–flavin conjugates are sequence-specific DNA-cleaving molecules,[7] and flavin–peptide conjugates are able to repair a cyclobutane uracil dimer incorporated into an oligonucleotide.[8] Here, we show that flavin–oligonucleotide conjugates selectively photocleave a double stranded DNA target present in the HIV-1 genome and display original DNA photosensitisation properties.

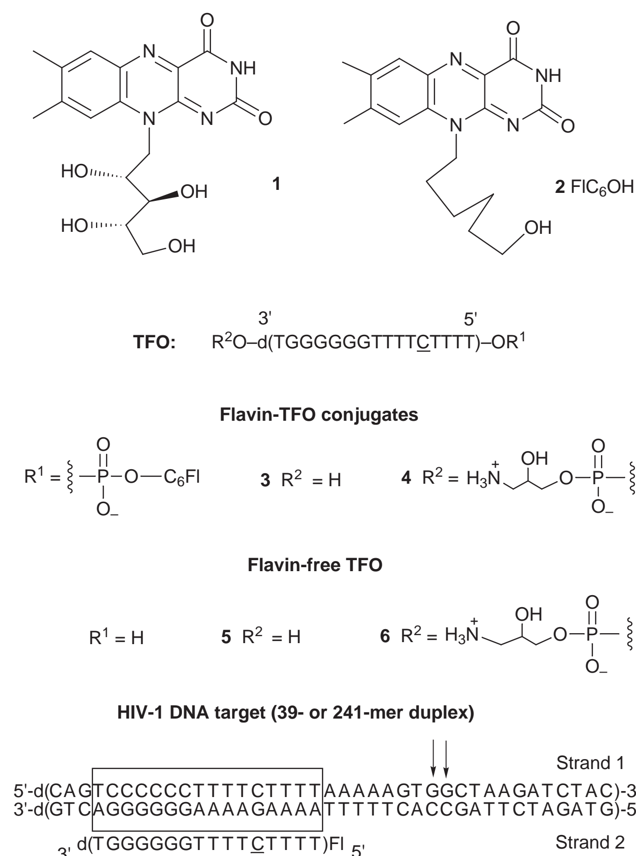

Fig. 1 Structures and sequences (phosphodiester linkages). d(C): 5-methyl-2′-deoxycytidine; DNA target: PPT sequence in a 39 or 241 base pair duplex present in the HIV-1 genome. The arrows indicate the sensitive GG doublet.

The flavin–TFO conjugates **3** and **4** (Fig. 1) were prepared as previously described.[9] The selected 16-mer TFO sequence has been previously shown to allow triple helix formation with duplexes containing the sixteen consecutive bases of the polypurine tract (PPT, Fig. 1) of the HIV-1 genome.[10] The target duplex was obtained from a 241-mer PCR fragment cloned into a pGEM-3Z plasmid. That the triple helix, formed upon hybridisation of the flavin–oligonucleotide conjugate **4** or the corresponding oligonucleotide **6** to the target duplex, was located as predicted was shown from footprinting experiments (data not shown). The protection of the whole PPT sequence of the 241 base pair fragment from hydrolysis by DNase I increased with increased amounts of both oligonucleotides. It should be noted that only the flavin–TFO conjugate **4** gave rise in the absence of irradiation to a large cleavage enhancement selectively at the guanine residue located 7 bases downstream of the triplex-duplex junction. This could be due to a local deformation of the duplex specifically induced by the flavin–TFO.

Triplexes were irradiated for 60 min with UV light and both strands of the target were analyzed by electrophoresis. The observed photocleavage was highly selective as it occurred exclusively on strand 1 and at the closest DNAse I-sensitive GG sequence, located 7 bases downstream of the triplex–duplex junction (Fig. 2, lane 13). No cleavage on strand 2 could be detected in spite of the presence of a GG sextuplet. With the free flavin **2**, alone (lane 7) or in the presence of the flavin-free TFO **6** (lane 11), the photocleavage occured at all G multiplets. No cleavage was observed with the flavin-free TFO (lane 9).

In all cases, irradiation was absolutely required as no reaction occured in the dark (lanes 6, 8, 10, 12). It is interesting to note that the flavin system is very photoreactive; a low intensity light source is able to induce the cleavage (365 nm, 5 mW cm^{-2}, 100 W UV or desk lamp). DNA cleavage was monitored by gel electrophoresis after removal of the major part of spermine by precipitation. Reaction yields were found to depend on the heating time before electrophoresis in the presence of urea (pH 8), suggesting that base damage was heat-labile. Whereas only 25% of the target was cleaved after 3 min heating at 90 °C, the yield was greater than 50% after 30 min heating. Further treatment with 1 M piperidine at 90 °C for 30 min did not increase this yield significantly.

To further characterize the reaction, we used 39-mer oligonucleotides as the target duplex containing the PPT sequence (Fig. 1). Again, a remarkably time- and light-dependent selective cleavage was obtained, with the labile site almost exclusively located at the 3′-guanine of the 5′-GG-3′

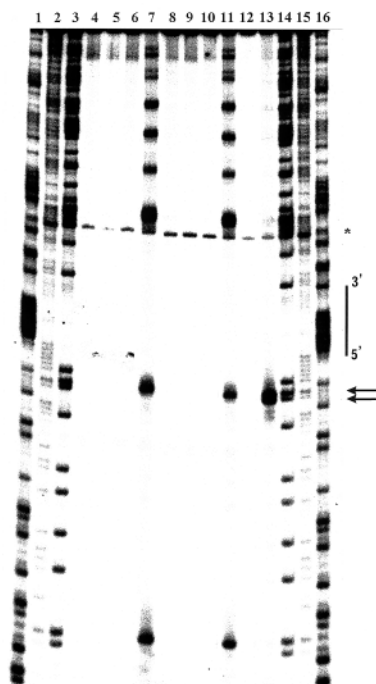

Fig. 2 Phosphoimager picture of a 12% denaturing polyacrylamide gel showing the cleavage products of a 241 base pair duplex DNA target (10 nM, Fig. 1), labeled on strand 1 at the 3′-end, alone (lanes 4,5) or incubated with 10 μM of the flavin **2** (lanes 6,7), the flavin-free TFO **6** (lanes 8,9), both **2** and **6** (lanes 10,11) or the flavin–TFO conjugate **4** (lanes 12, 13). Irradiated samples (lanes 5, 7, 9, 11, 13) are compared to dark samples (lanes 4, 6, 8, 10, 12). Sequencing reactions: T (lanes 1, 16), [G+A] (lanes 2, 15), G (lanes 3, 14). Hybridisation: overnight at room temperature, 20 mM Tris, pH 6.8, 5 mM $MgCl_2$, 0.25 mM spermine, 0.5 μg ml^{-1} of calf thymus DNA; irradiation (365 nm): 1 h at 4 °C, precipitation in EtOH and then heating in urea (7 M, pH 8, 10 mM Tris, 1 mM EDTA) at 90 °C for 5 min. The vertical line shows the presumed position of the flavin–TFO with the flavin moiety at the 5′-end. The arrows show the unique site of damage in the case of **4**. The starting DNA material contained one contaminant (asterisk).

sequence of the target (data not shown). Longer irradiation times also generated a minor amount of cleavage at the 5′-guanine. While alkaline treatment did not significantly affect gel profiles, treatment of the irradiated samples with the enzyme formamidopyrimidine-DNA glycosylase (Fpg) before electrophoresis revealed a second type of damage. The latter, most likely due to the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo),[11] was detected exclusively at the 5′-guanine of the same sensitive 5′-GG-3′ sequence (data not shown).

Similar results were obtained with the flavin–oligonucleotide conjugate **3**. These results provide the first indication of the potential of flavin–oligonucleotide conjugates as selective DNA photocleaving agents. Cleavage of a viral target occured at only one GG site among 26 G multiplets present in the duplex target (with a total of 111 G). It is likely that the observed DNA damage is the result of electron transfer from guanine to the photoexcited flavin acceptor,[12] in agreement with GG doublets being the most efficient donors.[13] In addition to this high selectivity, this system displays several original features which have yet to be understood: (i) both the 3′- and 5′-G of the GG doublet are damaged while previous studies indicated a higher sensitivity for the 5′-G,[13] and (ii) the reactive doublet is several bases away from the presumed flavin site. Whether the reaction is made possible by a local bending of the HIV DNA in the proximity of the PPT boundary, thus allowing a direct interaction between the flavin and the GG doublet (direct H-abstraction from the sugar or electron transfer), or by a long-range electron transfer[14] is a fascinating question which remains to be elucidated.

We thank Jacques Laval for the gift of Fpg, and financial support from Agence Nationale de la Recherche sur le SIDA (ANRS) is gratefully acknowledged.

Notes and references

1 K. J. Scanlon, Y. Ohta, H. Ishida, H. Kijima, T. Ohkawa, A. Kaminski, J. Tsai, G. Horng and M. Kashani-Sabet, *FASEB J.*, 1995, **9**, 1288; W. Roush, *Science*, 1997, **276**, 1192.

2 C. Hélène, *Anticancer Drug Des.*, 1991, **6**, 569; N. T. Thuong and C. Hélène, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 666; M. D. Frank-Kamenetskii and S. M. Mirkin, *Annu. Rev. Biochem.*, 1995, **64**, 65.

3 A. S. Boutorine, D. Brault, M. Takasugi, O. Delgado and C. Hélène, *J. Am. Chem. Soc.*, 1996, **118**, 9469; P. Bigey, G. Pratviel and B. Meunier, *J. Chem. Soc., Chem. Commun.*, 1985, 181. For reviews, see: J. Goodchild, *Bioconjugate Chem.*, 1990, **1**, 166; U. Englisch and D. Gauss, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 613; R. S. Varma, *Synlett.* 1993, 621.

4 P. Gaudu, D. Touati, V. Nivière and M. Fontecave, *J. Biol. Chem.*, 1994, **269**, 8182.

5 H. Kasai, Z. Yamaizumi, M. Berger and J. Cadet, *J. Am. Chem. Soc.*, 1992, **114**, 9692; K. Ito, S. Inoue, K. Yamamoto and S. Kawanishi, *J. Biol. Chem.*, 1993, **268**, 13221; K. Kino and I. Saito, *J. Am. Chem. Soc.*, 1998, **120**, 7373.

6 P. Burgstaller and M. Famulok, *J. Am. Chem. Soc.*, 1997, **119**, 1137.

7 M. Bouziane, C. Ketterlé, P. Helissey, P. Herfeld, M. Le Bret, S. Giorgi-Renault and C. Auclair, *Biochemistry*, 1995, **34**, 14051.

8 T. Carell and J. Butenandt, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1461.

9 C. Frier, J.-L. Décout and M. Fontecave, *J. Org. Chem.*, 1997, **62**, 3520.

10 C. Giovannangéli, M. Rougée, T. Garestier, N. T. Thuong and C. Hélène, *Proc. Natl. Acad. Sci. U.S.A.*, 1992, **89**, 8631; P. O. Ilyinski and R. C. Desrosiers, *EMBO J.*, 1998, **17**, 3766.

11 J. Tchou, H. Kasai, S. Shibutani, M.-H. Chung, J. Laval, A. P. Grollman and S. Nishimura, *Proc. Natl. Acad. Sci. U.S.A.*, 1991, **88**, 4680.

12 J. Cadet, M. Berger, G. W. Buchko, P. C. Joshi, S. Raoul and J.-L. Ravanat, *J. Am. Chem. Soc.*, 1994, **116**, 7403; G. W. Buchko, J. Cadet, B. Morin and M. Weinfeld, *Nucleic Acids Res.*, 1995, **19**, 3954.

13 H. Sugiyama and I. Saito, *J. Am. Chem. Soc.*, 1996, **118**, 7063.

14 U. Diederichsen, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2317; R. E. Holmlin, P. J. Dandliker and J. Barton, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2714; S. M. Gasper and G. B. Schuster, *J. Am. Chem. Soc.*, 1997, **119**, 12762.

Communication 8/06119J